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Phaeocollybia nigripes (*Agaricomycetes*), a new species from Brazil

Victor R.M. Coimbra[1]*, Tatiana B. Gibertoni[1] & Felipe Wartchow[2]

[1] *Universidade Federal de Pernambuco, Departamento de Micologia/CCB, Av. Prof. Nelson Chaves, s/n, CEP: 50670-901, Recife, PE, Brazil*

[2] *Universidade Federal da Paraíba, Departamento de Departamento de Sistemática e Ecologia, CEP: 58051-970, João Pessoa, PB, Brazil*

* Correspondence to: *vick_mat_coimbra@yahoo.com.br*

Abstract —*Phaeocollybia nigripes* is described from an Atlantic Forest fragment in Pernambuco, Northeast Brazil. It is characterized by a stipe that is uniformly slender with a slightly swollen base and dark grayish brown below to almost black at the apex, brown pileus, large basidiospores that average 12.4 × 6.6 µm, hyphoid to narrowly clavate cheilocystidia, and abundant clamp connections. The holotype of *P. megalospora* var. *megalospora*, the putatively most similar taxon, was examined. A key to the six taxa reported from Brazil is also presented.

Key words — *Agaricales*, *Hymenogastraceae*, neotropic, taxonomy

Introduction

Phaeocollybia R. Heim was originally described to accommodate taxa among the brown-spored *Agaricales* with smooth and moist to viscid pilei and long pseudorhizas (Heim 1931). Matheny et al. (2006), based on multilocus phylogenetic analyses, placed the genus as represented by *P. festiva* (Fr.) R. Heim in the *Hymenogastraceae* clade, but Kirk et al. (2008), continued to refer *Phaeocollybia* to *Cortinariaceae*. More recently, Petersen et al. (2010), also using molecular data, treated *P. festiva* and *P. dissiliens* A.H. Sm. & Trappe in the bolbitioid clade with *Bolbitius* Fr., *Panaeolus* (Fr.) Quél., and *Conocybe* Fayod.

Phaeocollybia has been rarely reported in Brazil. The few taxa known from this country were all reported from the Amazon region by Singer (1987), who cited *P. brasiliensis* I.A. Aguiar ex Singer, *P. elaeophylla* Singer, *P. flava* I.A. Aguiar ex Singer, *P. megalospora* I.A. Aguiar ex Singer var. *megalospora*, and *P. megalospora* var. *tetraspora* Singer.

In this paper, we describe a new species based on a collection from the Atlantic Forest of Pernambuco, Northeast Brazil, and provide a key to the Brazilian *Phaeocollybia* species.

Materials & methods

One basidioma was collected in an Atlantic Forest fragment called Ecological Reserve of Dois Irmãos (8°01′15″S 34°52′30″W), a 387.4 ha area comprising a remnant of Atlantic Forest and a public zoo in the urban perimeter of the municipality of Recife (Ribeiro et al. 2007, Souza et al. 2009).

Colors are coded according to Watling (1969). The key to species is based mainly on data given by Singer (1987). Basidiospore data terminology follows Tulloss et al. (1992) but based on 25 basidiospores measured from a single basidioma (Wartchow 2009). Abbreviations include **L**(**W**) = basidiospore length (width) average, Q = the length : width ratio range as determined from all measured basidiospores, and **Q** = the Q value averaged from all basidiospores measured.

Scanning electron microscopy (SEM) studies were conducted at the 'Laboratório de Microscopia Eletrônica' (DF/UFPE). Sections were removed from dried basidiomata and mounted directly on aluminum stubs using carbon adhesive tabs. The fragments were coated with 8–13 nm of gold using a sputter coater and examined with a scanning electron microscope.

Phaeocollybia nigripes Wartchow & V. Coimbra **sp. nov.** Figs. 1-6, 8

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Ab Phaeocollybia megalospora *var.* megalospora *differt stipe coloribus apice niger versus fuscus basin, cheilocystidiis tenui clavati vel cylindraceous et catenatus, et pileipellis hipodermio ex elementis pro ratione voluminosis.*

Holotype: Brazil, Pernambuco, Recife, Ecological Reserve of Dois Irmãos, 10.iii.2009 V.R.M. Coimbra, J.M. Baltazar & L. Trierveiler-Pereira s.n. (URM 82277, **holotype**).

Etymology: Latin for 'black foot,' in reference to the stipe color.

Pileus 11 × 10 mm, conic, reddish brown to dark brown ('Date brown 24'), surface smooth, margin slightly short-sulcate and involute. Lamellae adnexed, close, lamellulae present, dark grayish brown (slightly lighter than 'Drab 33') with dark gray edges (under 10× lens), edges entire. Veil not evident. Stipe 70 × 1.5–2 mm (upper portion apart from pseudorhiza 55 mm long), cylindrical above a swollen base, central, dark brown ('Cigar brown 16'), shiny metallic graphite-black at apex with a vinaceous base under intense light, surface smooth, hollow. Pseudorhiza thin, spiraling, ¼ of the stipe length.

Basidiospores (10–)10.8–14.5(–15) × (5.5–)6–7.3(–8) µm (**L** = 12.4 µm; **W** = 6.6 µm; Q = (1.69–)1.77–2.00(–2.29); **Q** = 1.88), amygdaliform to sometimes limoniform, thin-walled, surface minutely verrucose, brown. Basidia 24–33 × 9–11 µm, clavate, thin-walled, with prominent sterigmata, predominantly 2-sterigmate. Pleurocystidia absent. Cheilocystidia (13–)16–25 × 5–6 µm, narrowly clavate to hyphoid (located in terminal position of chains of short celled hyphae), forming dense tufts on the sterile edge, thin-walled, hyaline. Tibiiform diverticula abundant on pseudorrhizal pellis, 11–32 × 1.8–3 µm, narrowly lanceolate to subcapitate, sometimes with apical droplet, some with small lateral projections, hyaline, colorless, thin walled; on stipe more scattered

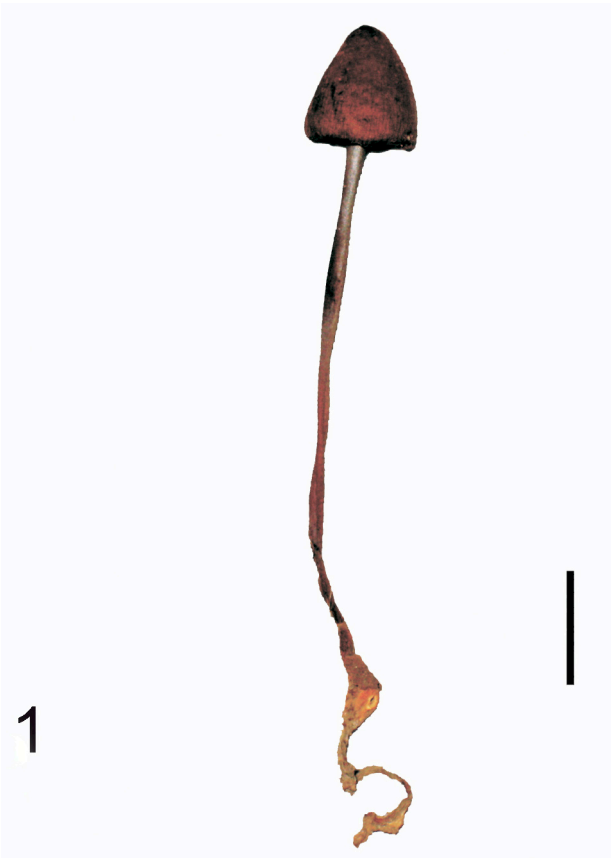
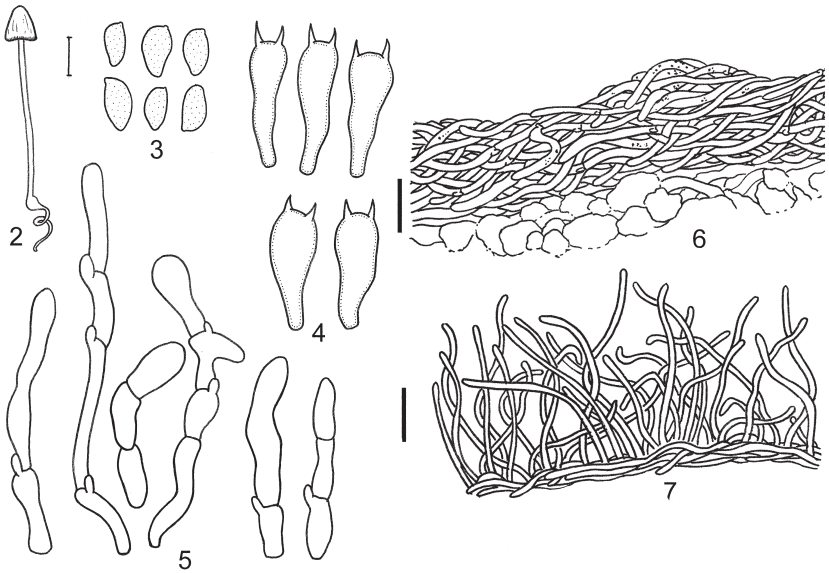


Fig. 1. *Phaeocollybia nigripes* (holotype). Basidioma. Scale bar = 10 mm.

and slightly smaller 11–16 × 2–3 µm, hyaline, thin walled. Pileipellis with periclinal to sometimes anticlinal hyphae of 3–6 µm wide, pigments encrusting, yellowish brown, showing some gelatinized zones; hypodermium somewhat pseudoparenchymatous, elements 20–27 × 13–18 µm, yellowish-brown. Lamellar trama regular and parallel, hyphae ranging 4.5–15.5 µm, thin-walled. Clamp connections abundant.

Ecology & distribution: Solitary on soil under dicotyledonous trees, including representatives of the ectomycorrhizal families (Smith & Read 2008) *Caesalpiniaceae* [*Senna* Mill., *Chamaecrista* (L.) Moench, *Dialium* L., *Hymenaea* L., *Sclerolobium* Vogel, *Zollernia* Maximil. & Nees], *Nyctaginaceae* (*Boerhavia*



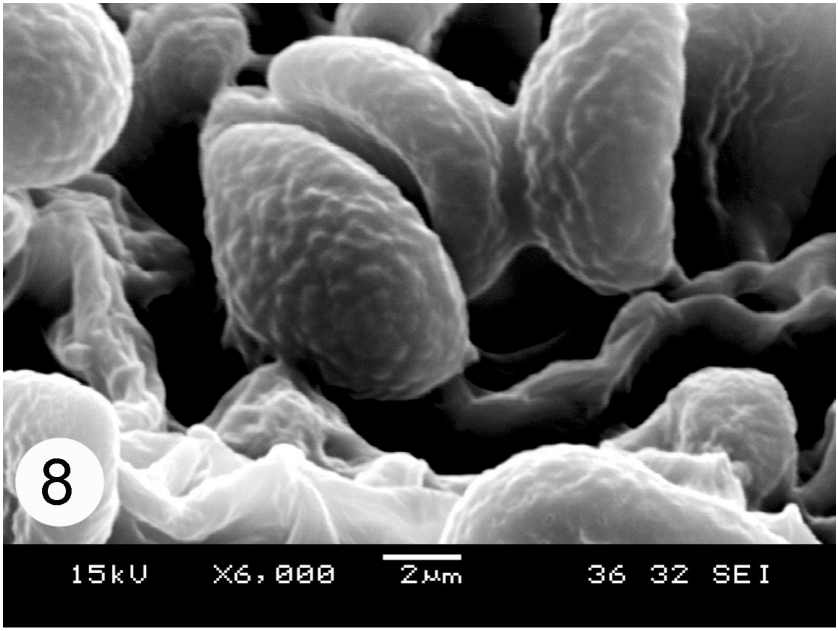
Figs. 2–6. ***Phaeocollybia nigripes*** (holotype): 2. Basidioma. 3. Basidiospores. 4. Basidia. 5. Hyphal elements of the lamella edge. 6. Pileipellis and adjacent inflated elements of the hypodermium. Fig. 7. ***P. megalospora var. megalospora*** (holotype): Pileipellis and adjacent hyphae of the hypodermium. Scale bars: 2 = 10 mm; 3–7 = 10 µm.

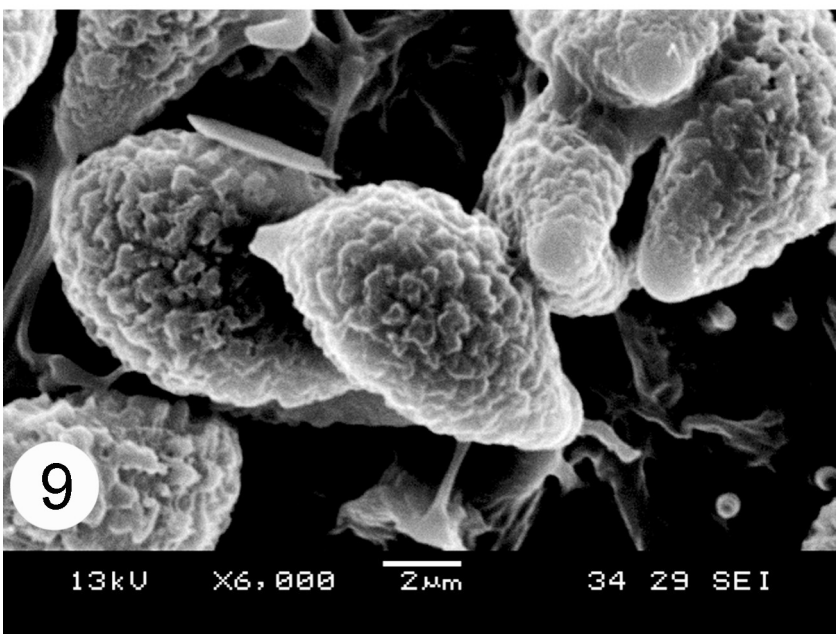
L., *Guapira* Aubl.) and *Polygonaceae* (*Coccoloba* P. Browne). Known only from the type locality in the Atlantic Forest of Northeast Brazil.

Additional specimen examined: ***Phaeocollybia megalospora*** var. ***megalospora***—BRAZIL. Amazonas, road Manaus-Caracaraí km 45, 11.vii.1977, R. Singer & I. Araujo, Singer B 10082 (INPA 102697, **holotype!**).

Remarks: The distinctive microscopic features of *P. nigripes* include basidiospores longer than 11 µm, predominantly 2-spored basidia, cylindric-hyphoid to narrowly clavate cheilocystidia, and a slightly pseudoparenchymatous hypodermium. The pseudorhiza of our new species was broken during collection.

Following the classification proposed by Bandala & Montoya (1994), *P. nigripes* would be referred to subgen. *Fibulophaeocollybia* Bandala & Montoya sect. *Subattenuatae* Singer based on the presence of clamp connections (most easily seen at the cheilocystidial bases) and limoniform basidiospores longer than 7 µm (Bandala & Montoya 1994). Norvell & Exeter (2009), who felt that infrageneric classification is premature in the absence of molecular analyses of the whole genus, suggested that cheilocystidial morphology might be a better





Figs. 8–9. SEM of basidiospores (photos by Sérgio Santos).
8. *Phaeocollybia nigripes*. 9. *P. megalospora*.

morphological character for identifying monophyletic groups below genus level in *Phaeocollybia*.

Phaeocollybia megalospora var. *megalospora*, characterized by Singer (1987) as also producing 2-sterigmate basidia and similar sized basidiospores, differs in its yellowish to brownish stipe, pileipellis with a gelatinized ixocutis and cutiform rather than pseudoparenchymatous hypodermium, ampullaceous cheilocystidia, and more strongly verrucose basidiospores (Singer 1987). We examined the holotype (Figs. 7, 9) of *P. megalospora*, which we believe is the same basidioma depicted in Singer (1987: Plate III, fig. 5), and noted that the stipe was apparently broken and lacked the pseudorhiza (which typically bears abundant tibiiform diverticula according to Norvell 1998), so that only one very small (<7 × 2 µm) tibiiform diverticulum was observed. The pileipellis comprised gelatinized erect, non-incrusted, 1–3.5 µm wide, yellowish hyphae with the hypodermium comprising hyphae ≤5 µm wide, radially oriented, and with dark brown vacuolar pigments (Fig. 7). The basidiospores are very similar in size 11–15 × (6–)6.5–8 µm (**L** = 12.9 µm; **W** = 7.2 µm; Q = (1.43–) 1.50–2.00(–2.14); **Q** = 1.80) but more strongly verrucose (Fig. 9) than those found in our species, supporting our conclusion that *P. megalospora* var. *megalospora* and *P. nigripes* are distinct species.

Another taxon with blackish tints in the basidioma is *P. singularis* E. Horak & Halling from Colombia (Horak & Halling 1991) and the Talamanca Mountains in Costa Rica (Norvell pers. corr.), but it obviously differs in the blackish lavender pileus, violaceous stipe, smaller (8–9.5 × 4.5–5 µm) basidiospores, and tibiiform cheilocystidia.

Phaeocollybia phaeogaleroides Norvell is another similar species with relatively large basidiospores. It shares with *P. nigripes* the dark brown pileus and stipe, clamp connections, and cylindrical cheilocystidia (Norvell 2002) but differs in the hygrophanous pileus, shorter narrower basidiospores 9–12(–13) × 5–6.5 µm (**L** = 10 µm; **W** = 5.8 µm), 4-spored basidia, and longer (≤ 80 µm) subcapitate cheilocystidia (Norvell 2002).

Phaeocollybia longistipitata Halling & E. Horak, *P. odorata* E. Horak, *P. pleurocystidiata* Norvell & Redhead, *P. singeri* Guzmán et al., and *P. tentaculata* E. Horak are other slender stiped taxa that are segregated primarily by their smaller basidiospores and lack of clamp connections (Horak 1977, Bandala et al. 1989, Norvell & Redhead 2000, Halling & Horak 2008).

Phaeocollybia columbiana Singer from tropical rain forest of Colombia also shares relatively large (10–12 × 6–7 µm) basidiospores. However, it differs in the spadiceous pileus with whitish centre, ochraceous brown stipe, ventricose cheilocystidia, and absence of clamp connections (Singer 1970, Horak 1977).

Phaeocollybia amazonica Singer, from the Bolivian Amazon, differs from *P. nigripes* macroscopically in pileus size (40–45 mm diam), ochraceous brown

to rusty lamellae, and cinnamon stipe and microscopically in basidiospore shape and larger (>30 × 6 µm) cheilocystidia (Singer 1961, 1970).

Other lowland Amazonian Brazilian taxa — *P. brasiliensis*, *P. elaeophylla*, *P. flava*, *P. megalospora* var. *tetraspora* — are easily segregated by their smaller basidiospores (Singer 1987).

Here we also address the ecological status of *Phaeocollybia nigripes*. Earlier studies implied *Phaeocollybia* as saprotrophic with the pseudorhizae arising from buried wood (Smith 1957; Smith & Trappe 1972). Subsequently Redhead & Malloch (1986), who studied the temperate *P. christinae* (Fr.) R. Heim and *P. jennyae* (P. Karst.) Romagn., concluded that *Phaeocollybia* is parasitic based on the successful excavation of a pseudorhiza connected to a senescent spruce rootlet. Norvell (1998), demonstrating that *P. redheadii* Norvell, *P. kauffmanii* (A.H. Sm.) Singer, and several other taxa present ectomycorrhizal mantles and Hartig-net in western hemlock and noble fir roots, emphasized that all Pacific Northwest North American *Phaeocollybia* species are found in forests with *Tsuga*, *Picea*, *Abies*, *Pseudotsuga*, *Lithocarpus*, *Quercus*, and/or *Pinus*, all ectomycorrhizal-forming genera (Smith & Read 2008). Most recently, Norvell & Exeter (2009) cite the presence of Hartig-net and high nitrogen isotope readings presented by the studied species as the main characters supporting the ectomycorrhizal nature of *Phaeocollybia* in the Pacific Northwest.

Neotropical phaeocollybias from Colombia and Costa Rica occur mostly under the ectomycorrhizal tree genus *Quercus* (Singer 1987, Horak & Halling 1991, Halling & Horak 2008), while others from lowland Amazon forests were found in "terra firme," a typical anectotrophic forest type according to Singer & Araújo (1979). In the case of *P. nigripes*, although no ectomycorrhizal connection was detected, representatives of *Caesalpiniaceae*, *Nyctaginaceae,* and *Polygonaceae* occurred in the collection area (Guedes 1998, Ferreira et al. 2007, Souza et al. 2009); these families include genera shown to have ectomycorrhizal associations in the tropical forests of Ecuador: e.g., *Coccoloba* (*Polygonaceae*) and *Guapira* (*Nyctaginaceae*) (Tedersoo et al. 2010).

Artificial key for the Brazilian taxa of *Phaeocollybia*

1. Basidiospores small, 5–5.5 × 4.5–5 µm; pileus yellow . . . . . *P. flava*
1'. Basidiospores longer, > 7 µm long; pileus with brown tints 2

2. Basidiospores ≤10–15 µm long; basidia mostly with two sterigmata 3
2'. Basidiospores with largest length not longer than 11.5 µm; basidia with four sterigmata (in *P. elaeophylla* 1–4 sterigmata) 4

3. Stipe yellowish to brownish towards the base; cheilocystidia ampullaceous; hypodermium cutiform made of hyphae 3–9 µm wide . . . . . *P. megalospora* var. *megalospora*

3'. Stipe dark brown with shiny metallic graphite-black apex; cheilocystidia narrowly clavate to hyphoid (located in terminal position of chains of short-celled hyphae); hypodermium pseudoparenchymatous made of elements of 20–27 × 13–18 µm ..... *P. nigripes*

4. Lamellae olive; basidiospores 8.5–10.3 × 4.5–6.5 µm, cheilocystidia heteromorphous 17–49 × 3.5–5.5 µm, sometimes tibiiform-like .... *P. elaeophylla*

4'. Lamellae with no olive color 5

5. Pileus striate; basidiospores (7–)7.5–8.3 × 4.7–5.5 µm; cheilocystidia ventricose frequently capitate (tibiiform?) 25–32 × 6–7.5 µm ..... *P. brasiliensis*

5'. Pileus not striate; basidiospores 9.5–11.5 × 6–7 µm, cheilocystidia clavate, cylindrical or utriform of 40–50 × 3.8–6.5µm .... *P. megalospora* var. *tetraspora*

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